IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

App. No.	:	10/516,864	Confirmation No. 8549
Applicant	:	HSIAO, Wen-Luan Wendy and WONG, Sze-Chuen	
Filed	:	June 27, 2003	
TC/A.U.	:	1634	
Examiner	:	Goldberg, J.A.	
Docket No.	:	32144183-000004	
Customer No.	:	51738	
Entitled	:	Plasma or Serum Marker and Process for Detection of Cancer	

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF WEN-LUAN WENDY HSIAO UNDER 37 CFR §1.132

I, Wen-Luan Wendy Hsiao, declare as follows:

1. I am at least 18 years of age and am competent in all respects to make the following statements.

2. I am a joint inventor for claims 1-29 currently pending in US Patent Application No. 10/516,864.

3. I have read and understand the above-referenced application and pending claims.

4. I am a person of ordinary skill in the art of biomarkers for detection of cancer including colorectal adenoma and colorectal carcinoma, see the attached *curriculum vitae* (Appendix A).

5. To the best of my knowledge, the present invention is the **first** demonstration of detecting colorectal adenoma and colorectal carcinoma by measuring beta-catenin RNA and DNA in blood serum and plasma.

6. The attached figure (Appendix B) contains the data described in paragraph 31 of the above referenced application. The results show that a 359 bp band was observed in all 15 serum DNA samples (FIG. 3a, lanes 1 to 15). Ten patients were tested with confirmed adenoma ranging from mild to severe dysplasia. A positive band was detected in 9 of 10 patients (FIG. 3b, lanes 1-10). The detection rate was 90%. The only negative case (FIG. 3b, lane 8) was amplifiable as it yielded positive 156 bp band after amplification with RET specific primers (FIG. 3d, lower panel, lane 13). PCR amplification of β-catenin was also performed on 10 healthy volunteer controls. None of the serum samples showed positive signals for β-catenin, while positive signals were clearly detected using RET specific primers (FIG. 3c, lanes 1 to 10; and 3D, lanes 1 10). In addition, a known positive carcinoma serum sample was carried out in parallel and showed the typical 359 bp band on the agarose gel (FIG. 3c, lane 11). Lanes 16 and 17, FIG. 3a are the positive and negative control for the PCR reaction. Lane 12 of FIG. 3c & FIG. 3d are the negative control for PCR reaction. These results demonstrate the ability of β-catenin to accurately detect colorectal carcinoma and adenoma in serum.

7. Many DNA and RNA detection methods are used reliably in the field of biomarkers including PCR amplification, microsatellite PCR, hybridization, fluorescent in situ hybridization (FISH), immunochemical staining, reverse-transcriptase PCR (RT-PCR), FACS, and sequencing among others. Given a known DNA sequence, one of ordinary skill in the art could generate the probes and primers required for DNA and RNA detection without ambiguity or undue experimentation.

8. Cesar Wong and I performed most of the work published in Wong et al. (Clinical Cancer Research, Vol. 10, pages 1613-7, March 2004) while Cesar Wong was working toward a Ph.D. degree under my supervision. Later, the work was published without my permission and without recognizing me in the publication. Thus, Wong is the work of the current inventors.

9. Beta-catenin is representative of many catenin/cadherins that increase expression in neoplastic tissues. If beta-catenin RNA and DNA are found in the plasma and serum, alpha-catenin and E-cadherin RNA and DNA will likewise be found in the serum and plasma.

I further declare that all statements made herein of my own knowledge are true and made on information believed to be true; further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of any application for which it is used.

Dated: January 15, 2008

Respectfully submitted,

By _____

Wen-Luan Wendy Hsiao
Professor of
Hong Kong Baptist University
Flat 19-A, Tower #1, Oscar by the Sea,
8 Pung Loi Road, Tseung Kwan O
Kowloon
Hong Kong
CN

APPENDIX A: CURRICULUM VITAE OF DR. HSIAO.

Prof HSIAO, Wen-Luan Wendy

Professor, Acting Coordinator, Full-time Programme Unit
Expert in Molecular Biology and Tumor Pathology

Prof HSIAO's main research interest is in gene regulation and cell signaling in multistage carcinogenesis, with emphasis on factors, agents, and drugs modulating expressions of oncogenes and tumor suppressor genes. Her early work on the interplay of tumor promoter TPA and ras oncogene has made a significant contribution toward the understanding of the role of tumor promoters in cancer development. Prof HSIAO's recent research has extended into the newly identified genes involved in the Wnt signaling pathway. The goal is to investigate their roles in tumor formation, and cell signaling in response to chemotherapeutic and preventing agents, including natural products.

In addition to her basic research programs, Prof HSIAO also takes keen interest in screening and delineating mechanisms underlying the actions of herbal medicines with cancer therapeutic and preventive potentials. Her specific focus is on non-toxic saponins and polysaccharide-peptides. Over the years, Prof HSIAO has developed various bioassays to assess the cytotoxicity environmental chemicals. During her tenure at University of California, Irvine, she has made a major contribution in developing in vitro assays to test non-genotoxic chemicals for the National Institute of Environmental Health Science, USA, under the National Toxicology Program. Currently, she is participating in a multi-disciplinary project to assess the environmental health impact of respirable atmospheric particles (PM-2.5) in Hong Kong. At present, Prof HSIAO is the author of over 70 publications in top ranking international journals, covering the fields of cancer and molecular biology, clinical pathology, environmental research, and herbal medicines. She is also the inventor of several patents and patent applications.

APPENDIX B: SERUM B-CATENIN RNA.